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Letter to the Editor

T CELL SUBPOPULATIONS IN B CELL CHRONIC LYMPHOCYTIC LEUKAEMIAS

Sir, In a recent paper Dickinson, George & Proctor (1983) compared two techniques for enriching T cells from patients with B cell type chronic lymphocytic leukaemias (B-CLL) and controls, namely sheep red blood cell (SRBC) rosetting and nylon wool column elution. In particular they evaluated the effects of these methods on relative proportions of OKT4 and OKT8 positive supopulations. These authors suggested that the two techniques produce significantly different subpopulations of T cells and, on the basis of this statement, cast some doubt on the extent of the increase in OKT8 positive T cells observed in these patients (Hermann *et al.*, 1982; Mills, Worman & Cawley, 1982; Semenzato *et al.*, 1983). We would like to comment on this paper.

At first a few methodological observations are in order. It is important to evaluate not only percentages but also the absolute numbers of populations we are dealing with. Dickinson and co-workers' enriched T cells obtained by the SRBC rosetting method accounted for 51·7% and 55·1% of peripheral blood lymphocytes of controls and CLL, respectively. In our opinion there is no sense in studying samples with such a low recovery of T cells after purification. In fact, they have evaluated only half of total T cells of their patients, and in the other half they could have selectively missed discrete cell subsets.

A second major criticism of Dickinson and co-workers' paper is their failure to examine the nylon wool adherent and E depleted fractions for residual $OKT4^+$ or $OKT8^+$ cells. We have examined E^- preparations from normal individuals and B-CLL patients and found $<2\%$ $OKT4^+$ and $<5\%$ $OKT8^+$ cells. In a limited number ($n=3$) of nylon wool adherent peripheral blood mononuclear cells from normal subjects, we found $<2\%$ $OKT4^+$ and up to 10% $OKT8^+$ cells. These findings suggest that E rosetting does not enrich $OKT8^+$ cells and that nylon wool elution may enrich for $OKT4^+$ cells. Supporting the above results, data in the literature reported that selective T cell subpopulations are missed by nylon wool elution (Tada *et al.*, 1978; Henry, 1980).

The third point concerns the value of the OKT4/OKT8 ratio reported by Dickinson *et al.* (1983), which is unusually low (1·23). In contrast, we have always found ratios of approximately 2 for both normal unfractionated and E enriched peripheral blood mononuclear cells, and this figure is in close agreement with the majority of values reported in the literature (Davis, 1981; Platsoucas *et al.*, 1982; Mittelman *et al.*, 1984; Pizzolo *et al.*, 1983; Sabbe *et al.*, 1983; Foa *et al.*, 1984). The previously considered selective loss of T cells in their separation procedures may account for the low value. In addition, in order to lyse the SRBC pellet Dickinson *et al.* (1983) used distilled water. Avoiding this procedure is really important. In fact, data from our labs and by other authors (B. Doerken, Heidelberg, personal communication) point out that the lysis with distilled water strongly affects the ratio within E enriched populations. In particular, the expression of OKT4 determinant is easily damaged (as much as 30% of this subset).

Finally, Dickinson *et al.* (1983) chose normal healthy laboratory personel (mean age 28 years) to establish control ranges. Since a difference has been demonstrated in the pattern of reactivity of OKT4 and OKT8 monoclonal antibodies according to the age (Nagel *et al.*, 1983; Burton *et al.*, 1983), the absolute necessity of selecting age matched controls is emphasized.

In order to over come the problem of the cell purification in CLL patients, we have used a double staining technique with Leu 3 (OKT4 equivalent) fluorescein conjugated and Leu 2 (OKT8 equivalent) phycoerythrin conjugated monoclonal antibodies, to evaluate simultaneously the frequency of T cell subsets in peripheral blood and E enriched fractions of eight patients with

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Table 1. OKT4/OKT8 ratio in peripheral blood lymphocytes (PBL) and enriched T cell populations from patients with CLL and controls (mean ± s.e.)

OKT4/OKT8 ratio	Controls* ($n=5$)	CLL patients† ($n=8$)
PBL	2·44 ± 0·6	1·10 ± 0·28
Enriched T cells	2·52 ± 0·7	1·02 ± 0·21
P	NS	NS

* Age matched controls.

† One patient stage I, two patients stage II, three patients stage III, two patients stage IV.

NS = not significant (Student's *t*-test).

B-CLL and of five aged matched controls. As shown in the accompanying Table 1, we constantly found similar results.

Additional evidence that our method of enriching T cells does not influence the balance of T cell subsets is provided by Ziegler *et al.* (1981). They found that T cells from CLL patients purified by B cell elimination with anti-B cell monoclonal antibody and complement without SRBC rosetting, expressed increased numbers of cells with suppressor phenotype.

In the introduction of their paper, Dickinson *et al.* (1983) stated that contradictory results exist in the literature concerning T cell subsets in B-CLL patients. The matter has been recently reviewed by Foa *et al.* (1984). Apart from the fact that Matutes *et al.* (1981) found a reduction of $OKT4^+$ cells and not an increase as quoted by Dickinson *et al.* (1983), there is in fact general agreement about the surface phenotype of T cells in B-CLL patients, that is, reduced percentages but normal or increased absolute numbers (according to the degree of T lymphocytosis usually present in these cases) of $OKT4^+$ lymphocytes and increased numbers, both in percentages and absolute terms, of $OKT8^+$ cells. These date have been extensively confirmed by other groups around the world (Ziegler *et al.*, 1981; Davis, 1981; Platsoucas *et al.*, 1982; Mittelman *et al.*, 1984; Pizzolo *et al.*, 1983; Sabbe *et al.*, 1983; Foa *et al.*, 1984) and not only in our labs (Herrmann 1982; Mills & Cawley, 1982; Semenzato *et al.*, 1983) and the reason for this imbalance in the blood of these patients has been recently explained by a redistribution of $OKT4^+$ lymphocytes. In particular Pizzolo *et al.* (1983) demonstrated a clear predominance of T cell exhibiting the inducer phenotype (Leu 3^+) in the bone marrow of these cases. The presence of increased numbers of $OKT8^+$ cells in peripheral blood of these patients is also substantiated by increased suppressor cell function reported in B-CLL patients (Kay, 1981; Semenzato *et al.*, 1981; Herrmann *et al.*, 1983).

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